THE EFFECT OF SPECIFIC VACCINES ON RAT TYPHOID.*†

EDNA STEINHARDT AND THOMAS FLOURNOY.

(From the Laboratory of Hygiene, University of Michigan, and the Pathological Laboratory, Bellevue Hospital, New York.)

It is impossible to produce typhoid fever in small laboratory animals by feeding them with typhoid bacilli; therefore the immunity produced by specific vaccines has always been tested by subcutaneous or intraperitoneal inoculations of the living culture. These methods, however, do not reproduce a disease at all comparable to human typhoid.

Rats and mice, on the contrary, when fed with certain of the paratyphoid group contract a disease which closely resembles typhoid in man. This fact has been taken advantage of by a number of workers for a comparative study of the specific vaccines. Chief among these investigators are Loeffler, Marks, and recently Bruckner.

Loeffler,[1] working with his culture of *B. typhimurium* on field mice, found that all subcutaneous inoculations failed to protect against subsequent feeding, but in a few instances he apparently secured protection from previous feeding with dead or living cultures.

Marks,[2] working with the same culture on mice, was however unable to immunize his animals against subsequent feeding. More recently Bruckner[3] has tested the local and general immunity in white mice to paratyphoid bacillus *B.* He was able by continued previous feeding of small doses of living cultures to protect them against subsequent subcutaneous injections.

Metchnikoff and Besredka[4] have just published their work on

* Received for publication May 15, 1911.

† We wish to thank Dr. Park, director of the Research Laboratory, Board of Health of New York, for his courtesy in permitting us to complete the work in his laboratory.

[1] *Festschrift*, Bd. 1.

[2] *Arb. aus dem König. Inst. f. Exp. Therap.*, Frankfort, 1908, 4, p. 37.

[3] *Ztschr. f. Immunitäts.*, 1 Orig., 1911, 8, p. 434.

[4] *Ann. de l'Inst. Pasteur*, 1911, 25, p. 193.

typhoid in chimpanzees and gibbons. They were able to reproduce the disease in these animals by feeding them living cultures of human typhoid; but specific vaccines and previous feeding with dead bacilli failed completely to protect their animals from oral infection. They stated that the immunity produced by the subcutaneous or intraperitoneal inoculations of the specific vaccines is local, and has no effect on the infecting organisms that gain entrance to the body through the intestinal wall, reaching conclusions directly opposed to those of Bruckner, Calmette, and others.

Our experiments, which have been carried on for the last two years, were begun on white mice, using the commercial Danysz virus, one of the Gaertner group, as the test organism. Cultures killed by heat, Vaughan's residue, and protective inoculations of immune serum were tested. The feeding of dead bacilli caused death in mice, as the Danysz bacillus has a thermostabile endotoxin. All the injections were given subcutaneously, to make them more comparable with the method of inoculation of human beings. When later the vaccinated mice were fed with living cultures, no protection was shown; sickness, usually fatal, occurred exactly as in the untreated mice.

TABLE 1.

MICE INOCULATED SUBCUTANEOUSLY OR FED WITH DEAD BACILLI LATER TESTED AGAINST FEEDING WITH LIVE CULTURES.

White Mouse	Previous Treatment	Doses	Number of Days' Interval before Feeding with Live Culture	Treatment	Result
No. 1..	None	None	None	.....	Sick—died in 3 days
No. 2..	None	None	None	.....	Sick—died in 7 days
No. 3..	None	None	None	.....	Sick—recovered
No. 4...	Culture killed by heat	1–200 cult. subcut.	6 days	.....	Sick—died in 5 days
No. 5...	Culture killed by heat	Ditto	6 days	.....	Sick—died in 10 days
No. 6...	Culture killed by heat	Ditto	6 days	.....	Sick—recovered
No. 7...	Immune serum (rabbit)	¼ c.c. ser. subcut.	None	2 days later ¼ c.c. ser. subcut.	Sick—died in 23 days
No. 8...	Ditto	Ditto	None	Ditto	Sick—recovered
No. 9...	Ditto	Ditto	None	Ditto	Sick—recovered
No. 10..	Vaughan's residue	2.5 mg. subcut.	6 days	.....	Sick—died in 3 days
No. 11..	Vaughan's residue	2.5 mg. subcut.	6 days	.....	Sick—died in 4 days
No. 12..	Vaughan's residue	2.5 mg. subcut.	6 days	.....	Sick—died in 15 days
No. 13..	Vaughan's residue	2.5 mg. subcut. two doses at 4 days' interval	2 days	.....	Sick—died in 9 days
No. 14..	Vaughan's residue	Ditto	2 days	.....	Sick—died in 15 days

Results.—Previous vaccination with cultures killed by heat or Vaughan's residue failed to protect mice against subsequent feeding of live cultures. There was an apparent protection with immune serum but this was not borne out on repeating the experiments as shown in Table 2. All the vaccinations were repeated, giving the same results.

TABLE 2.

MICE RESISTANT FROM TABLE 1, RE-FED LIVE CULTURES—ALSO SERUM AND VACCINE TESTS REPEATED.

Mouse	Previous Treatment	Feeding of Live Cultures	Number of Days' Interval before Feeding with Live Culture	Result
No. 1.....	None	None	None	Sick—died in 2 days
No. 2.....	None	None	None	Sick—died in 7 days
No. 3.....	None	None	None	Sick—recovered
No. 4.....	None	Once—sick, recovered	45 days	Sick—died in 6 days
No. 5.....	Immune serum 2 doses	Once—sick, recovered	45 days	Sick—died in 9 days
No. 6.....	Immune serum 2 doses	Once—sick, recovered	45 days	Sick—died in 10 days
No. 7.....	Culture killed by heat 6 days later	Once—sick, recovered	45 days	Sick—died in 9 days
No. 8.....	Vaughan's residue—2.5 mg.	None	4 days	Sick—died in 2 days
No. 9.....	Ditto	None	4 days	Sick—died in 9 days
No. 10.....	Killed cultures 1 dose	None	4 days	Sick—died in 6 days
No. 11.....	Immune serum ½ c.c. subcut.	None	None	Sick—died in 7 days
No. 12.....	Ditto	None	None	Sick—died in 9 days
No. 13.....	Ditto	None	None	Sick—died in 11 days

Note.—The feeding of mice with dead bacilli caused their death, also subcutaneous inoculations of dead bacilli were often toxic. Vaughan's residue inoculated subcutaneously failed to protect mice from later intraperitoneal inoculations of the bacilli, although it did protect guinea-pigs thus treated from similar injections.

Method.—An emulsion was made from agar cultures of the Danysz bacillus. Small cubes of stale bread were moistened with 1 c.c. each of this emulsion. The mice, which had been kept without food for 12 to 24 hours, were separated, each receiving one cube of the infected bread. The next day after each one had eaten the bread, the mice were returned to a common receptacle. Autopsies showed congestion of the spleen, liver, and of the intestinal mucous membrane, and usually enlargement of Peyer's patches. Cultures were recovered from spleen and heart's blood in most cases. The feeding of all the treated and untreated mice was done at the same time, with the same emulsion, so that each

set acted as a standard of comparison for the others. The same method was used in all the following experiments except that the dose of the emulsion was increased for the rats.

After an interval, the mice that recovered were re-fed with the living culture and all contracted the disease again, showing that no immunity had been established even by a previous attack, thus differing from the results of Loeffler, who worked on field mice and with another organism; but agreeing with the results of Marks.

Results.—There was no apparent protection by the use of any specific method of vaccination; not even a previous attack of the disease gave rise to immunity in mice. The protection with the serum shown in Table 1 could not be reproduced, although the experiment was repeated a number of times. The individual resistance to the feeding plays a very important part as shown by the untreated mice, in which one died the second day after feeding and the other recovered, the only recovery in this series of 13.

Rats being less susceptible than mice, the experiments were repeated upon them. The vaccines used were cultures killed by heat, Vaughan's residue, sensitized bacilli (Besredka's vaccine), and preliminary feeding with small doses of the living culture. The technic was the same as that used for the mice. The animals injected subcutaneously with Vaughan's residue and those injected with the sensitized bacilli were not protected against later feedings of living cultures.

The rats injected with cultures killed by heat when fed later with living cultures all contracted the disease, but one-third recovered, whilst the disease was uniformly fatal for the rats vaccinated by other methods and the untreated controls.

The rats given preliminary small doses of living culture when fed later large doses of the living culture—the same dose that was uniformly fatal for the controls—were not even ill, complete protection having been established.

Results.—Previous vaccinations with sensitized bacilli did not protect against subsequent feeding with living culture. All the rats died, as did the controls, but in the rats vaccinated with cultures killed by heat, one-third were protected from death,

TABLE 3.

THE EFFECT OF FEEDING WITH LARGE DOSES OF LIVE CULTURE ON UNTREATED AND TREATED RATS.

Rat	Previous Treatment	Interval before Feeding with Live Culture	Result
No. 1. White control	None	None	Sick—died 6 days
No. 2. White control	None	None	Sick—died 6 days
No. 3. White control	None	None	Sick—died 27 days
No. 4. Gray and white control. . .	None	None	Sick—died 6 days
No. 5. Gray and white control . . .	*Sensitized bacilli* (Besredka's vaccine) 1/5 c.c. cult. subcut.	40 days	Sick—died 6 days
No. 6. Gray and white control. . . .	Ditto	40 days	Sick—died 6 days
No. 7. Gray and white control . . .	Ditto	40 days	Sick—died 9 days
No. 8. White control	*Sensitized bacilli* (Besredka's vaccine) 1/100 c.c. cult. subcut.	40 days	Sick—died 6 days
No. 9. White control	Ditto	40 days	Sick—died 9 days
No. 10. White control	Ditto	40 days	Sick—died 12 days
No. 11. White control	*Culture killed by heat* 1/1000 agar cult. subcut., 7 days later 1/1000 agar cult. subcut.	41 days	Sick—died 6 days
No. 12. White control	Ditto	41 days	Sick—died 10 days
No. 13. White control	Ditto	41 days	Sick—recovered
No. 14. White control	*Culture killed by heat* 1/1000 agar cult. subcut., 7 days later 1/100 agar cult. subcut.	41 days	Sick—died 6 days
No. 15. White control	Ditto	41 days	Sick—died 6 days
No. 16. Black and white.	Ditto	41 days	Sick—recovered
No. 17. Gray.	*Small doses live cult.* 1/2 agar cult. —not sick	22 days	Not sick
No. 18. Gray.	Ditto	22 days	Not sick
No. 19. Gray.	*Small doses live cult.* 1 agar cult. —not sick	22 days	Not sick
No. 20. Gray.	Ditto	22 days	Not sick
No. 21. White.	*Small doses live cult.* 1/10 agar cult.—not sick	22 days	Not sick
No. 22. White.	Ditto	22 days	Not sick
No. 23. White.	*Small doses live cult.* 2/5 agar cult. —not sick	22 days	Not sick
No. 24. White.	*Small doses live cult.* 2/5 agar cult. —died in 10 days		
No. 25. White.	*Small doses live cult.* 1/10 agar cult. —not sick	69 days	Not sick
No. 26. White.	Ditto	69 days	Not sick
No. 27. White.	Ditto	69 days	Not sick
No. 28. White.	Ditto	69 days	Not sick
No. 29. White.	Vaughan's residue 3 subcut. inoculations at 4 days' interval, followed in 33 days by feeding of 1/10 agar cult.	69 days	Not sick
No. 30. White.	Ditto	69 days	Not sick
No. 31. White.	Ditto	69 days	Not sick
No. 32. White.	Ditto	69 days	Not sick
No. 33. White.	*Sensitized bacilli* 1/4 agar slant subcut., 14 days later 1/6 agar slant subcut., 7 days later fed 1/10 live culture—not sick	69 days	Not sick
No. 34. White.	Ditto	69 days	Not sick
No. 35. White	Ditto	69 days	Not sick
No. 36. White.	Ditto	69 days	Not sick
No. 37. White.	*Culture killed by heat* 1/10 c.c. killed culture (5,000,000) subcut., 4 days later 2/10 c.c. killed culture subcut., 6 days later 4/10 c.c. killed culture subcut., 27 days later fed 1/10 live culture—not sick	69 days	Not sick
No. 38. White.	Ditto	69 days	Not sick
No. 39. White.	Ditto	69 days	Not sick

although all contracted the disease. The best protection, however, was afforded by small preliminary feedings of living cultures, as in the rats thus treated none showed any signs of sickness on subsequent feeding.

The rats which had resisted the feeding with large doses of the living culture, after an interval were tested as to their immunity against intraperitoneal inoculations. Although the results were not uniform, usually there was an increased resistance established, which either caused a delayed death, or, in a few cases, recovery. This increased resistance was as great in those rats which had only been fed several times with living cultures as in those which had been previously vaccinated and then fed with small doses of living cultures followed later by larger ones. The rats vaccinated subcutaneously did not show any greater intraperitoneal resistance than those treated by mouth.

TABLE 4.

THE EFFECT OF INTRAPERITONEAL INOCULATIONS ON RATS IMMUNE TO FEEDING OF LIVE CULTURES.

Rat	Previous Treatment	Number of Days Interval	Intraperitoneal Inoculation Dose	Result
No. 1. White..	None	None	½ agar culture	Died in 2 days
No. 2. White..	1 dose sensitized bacilli subcut. 14 days later repeated, 7 days later fed small dose live culture, 69 days later fed large dose live cult. — not sick (controls died)	30 days	½ agar culture	Died in 4 days
No. 3. White..	Fed small doses live culture—not sick, 69 days later fed large dose cultures —no sickness developed (controls died)	30 days	½ agar culture	Died in 8 days
No. 4. White..	None	None	$\frac{1}{10}$ agar culture	Died in 7 days
No. 5. White..	The same as for Rat No. 2	30 days	$\frac{1}{10}$ agar culture	Sick, recovered
No. 6. White..	The same as for Rat No. 3	30 days	$\frac{1}{10}$ agar culture	Sick, recovered
No. 7. White..	None	None	$\frac{1}{100}$ agar culture	Died in 7 days
No. 8. White..	The same as for Rat No. 2	30 days	$\frac{1}{100}$ agar culture	Sick, recovered

Result.—All the rats previously fed with live culture, whether vaccinated subcutaneously or not, showed an increased resistance to subsequent intraperitoneal inoculations, the subcutaneous vaccinations not apparently increasing this resistance.

Note.—The growth of culture on agar slant was more abundant than in Table 4, as a $\frac{1}{2}$ agar culture killed here in 18 hours, while in Table 4 it took two days.

Result.—As in Table 4 a resistance to intraperitoneal injection was shown to multiple lethal doses, except for one rat, which,

although immune to the feeding of living cultures, showed no increased resistance to intraperitoneal inoculations.

TABLE 5.

TESTING OF INTRAPERITONEAL RESISTANCE TO MULTIPLE LETHAL DOSES IN RATS IMMUNE TO FEEDING OF LIVE CULTURES.

Rat	Previous Treatment	Number of Days' Interval	Intraperitoneal Inoculation	Result
No. 1.....	None	None	½ agar culture	Died in 18 hrs.
No. 2.....	3 subcut. inoc. of Vaughan's residue (5 mg.) at 4 days' interval, 33 days after last inoculation fed small dose of live culture—not sick (controls not sick), 69 days after feeding fed large dose culture—not sick. (controls died)	35	½ agar culture	Died in 4 days
No. 3.....	$\frac{1}{10}$ c.c. killed culture subcut. inject. (ca. 5,000,000) bact., 4 days later $\frac{2}{10}$ of killed culture, 6 days later $\frac{4}{10}$ of killed culture, 27 days after last injection fed small dose live culture—not sick (controls not sick), 69 days after this feeding fed a large dose culture—not sick (controls died)	35	½ agar culture	Died in 4 days
No. 4.....	Fed small dose of live culture—slightly sick, 22 days later fed large dose live culture—not sick (controls died)	35	½ agar culture	Died in 18 hrs.
No. 5.....	None	None	1 agar culture	Died in 18 hrs.
No. 6.....	The same as for Rat No. 2	35	1 agar culture	Died in 3 days
No. 7.....	The same as for Rat No. 3	35	1 agar culturee	Died in 5 days
No. 8.....	The same as for Rat No. 4	35	1 agar culture	Sick, recovered
No. 9.....	The same as for Rat No. 2	35	2 agar cultures	Died in 18 hrs.
No. 10.....	The same as for Rat No. 3	35	2 agar cultures	Sick, recovered

Results.—In white mice the vaccination with cultures killed by heat, with Vaughan's residue, the treatment with immune serum, or previous feeding with live cultures failed to protect from subsequent feeding of large doses of living cultures.

In rats vaccination with sensitized bacilli gave no protection against subsequent feeding of large doses of living cultures.

Vaccination with cultures killed by heat saved one-third of the animals from death.

Preliminary feeding with small doses of living cultures gave complete protection against subsequent feeding of large doses of living cultures—doses which were uniformly fatal to the untreated control animals.

Conclusions.—In this work our results vary by varying the experimental animals. In all questions of immunity we think not alone the method of producing immunity but also the species and even

the individuals form a great factor, as well as the organism being tested.

From our results on rats we believe that, at least for them, the immunity produced by feeding is a general and not a local one, agreeing with Calmette, Bruckner, and others.

Thus far, we have been unable to produce a complete immunity against oral infection by subcutaneous inoculations, but the results with the cultures killed by heat were encouraging.

Further experiments with rats on vaccination, feeding with dead bacilli, specificity of the immunity, and treatment, specific and otherwise, are now being carried on, and the results will be given later.